Experimental Data Compiled November 2008

Recently a recombinant form of N-terminally truncated galectin-3, consisting of amino acids 7-143 of SEQ ID NO: 1, was produced. Using fluorescence polarization, the relative binding of the following to lacto-*N*-neotetraose (LNnT) was compared:

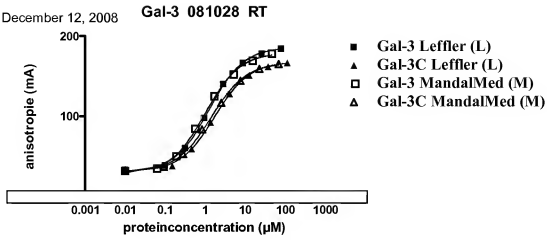
(i) N-terminally truncated galectin-3 consisting of amino acid residues 1-143 of SEQ ID NO:1, open triangle, MandalMed's laboratory

(ii) N-terminally truncated galectin-3 consisting of amino acid residues 7-143 of SEQ ID NO:1, closed triangle, Dr. Hakon Leffler's laboratory

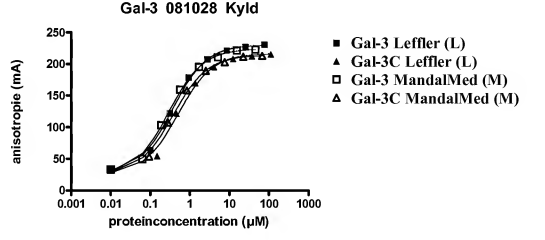
(iii) intact recombinant galectin-3 produced in Dr. Hakon Leffler's laboratory consisting of SEQ ID NO:3, closed square and

(iv) intact recombinant galectin-3 produced in MandalMed's also corresponding to SEQ ID NO:3, open square.

The data as shown in Figure 1 illustrates that the carbohydrate binding behavior of the two N-terminally truncated galectin-3 molecules was very similar. Here, the carbohydrate binding affinity of the *N*-terminally truncated molecules was less (greater dissociation constant Kd; Kd = [A][B] / [AB]) than that of the intact galectin-3.



	Gal-3 (L iii)	Gal-3C (L ii)	Gal-3 (M iv)	Gal-3C (M i)
A0	29.06	29.61	27.37	30.05
AMAX	186.6	168.4	179.6	166.9
KD	1.239	1.708	0.9951	1.405



	Gal-3 (L iii)	Gal-3C (L ii)	Gal-3 (M iv)	Gal-3C (M i)
A0	24.86	23.82	21.74	23.43
AMAX	230.9	216.8	224.4	214.2
KD	0.3620	0.5019	0.2896	0.3754

Fig 1. Fluorescence polarization analysis of binding of galectin-3 and two different N-terminally truncated galectin-3 molecules to fluorescently labeled lacto-*N*-neotetraose at room temperature (top) and 4°C (kyld; bottom). The difference in the affinities of the truncated galectin-3 molecules that differed in length by six amino acids mirrored the differences between the affinities of the two preparations of galectin-3, that had the identical structure but

The data above confirms that N-terminally truncated galectin-3 molecules that contain the entire carbohydrate recognition domain and lack that portion of the *N*-terminal domain required for multimerization would effectively and essentially equally block the cross-linking activity of galectin-3. This functional definition of "galectin-3C" is illustrated in Figure 2.

Fig. 2. Illustrates (top) galectin-3 with its carbohydrate recognition and *N*-terminal domains, (middle) two galectin-3 molecules dimerized through their *N*-terminal domains, and (bottom) galectin-3C lacking the N-terminal domain and unable to multimerize but retaining carbohydrate binding ability.

References

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2. Yang RY, Hill PN, Hsu DK, Liu FT. Role of the carboxyl-terminal lectin domain in self-association of galectin-3. Biochemistry 1998;37(12):4086-92.

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